CXLVII. THE RELATION OF THE COLOUR AND VITAMIN A CONTENT OF BUTTER TO THE NATURE OF THE RATION FED.

I. INFLUENCE OF THE RATION ON THE YELLOW COLOUR OF THE BUTTER.

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II. THE CAROTENOID AND VITAMIN A CONTENTS OF THE BUTTER.

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I. INFLUENCE OF THE RATION ON THE YELLOW COLOUR OF THE BUTTER.

UNDER the normal system of feeding dairy cows in winter in this country a most noticeable feature is the pale colour of the milk, a fact greatly emphasised in the butter made from it. The intensity of the yellow colour of the milk fat is positively correlated [Watson *et al.*, 1933] with the carotene content of the butter and also with its vitamin A content. The inclusion in the ration of some foodstuff capable of increasing the yellow colour of the milk fat is therefore of considerable importance. The oil cakes and cereals in common use are incapable of bringing about this result, for which the use of some foodstuff rich in carotene is needed. Carrots themselves may be used, but there are difficulties in the way of their adoption in general practice. In one case examined the milk of the herd produced a butter with a total yellow colour of 3·2 Lovibond units[1] which was raised to 7·2 units by feeding about 10 lb. of carrots per head daily.

In an experiment carried out in the winter of 1931–32 [Watson *et al.*, 1933] and also reported in this *Journal* [Gillam *et al.*, 1933], it was shown that artificially dried grass was a useful foodstuff in this regard when it formed 75 % of the total concentrated foodstuffs fed. This work has been extended in 1932–33 to test the value of different methods of conservation of pasture grass and their effect, in the ration, on the colour of butter.

[1] The yellow colour is stated in all cases as the Lovibond yellow units corresponding to a 1 cm. thickness of the fat. For the measurement it is diluted with three times its volume of light petroleum, the actual reading being multiplied by four.

In the first experiment four groups of Shorthorn cows were used. All groups received a basal ration of hay and roots. Group D1 acted as control and received a normal winter ration of concentrated foodstuffs, balanced for milk production, at the rate of $3\frac{1}{2}$ lb. per gallon of milk produced. Groups E1, E2 and E3 received 10 %, 25 % and 50 % respectively of their concentrate ration in the form of artificially dried grass, the mixture being so adjusted that the same amounts of nutrients (starch equivalent and protein equivalent) were fed per gallon of milk to all four groups. The values obtained for the total yellow colour of the butter are given in Table I, and are shown in Fig. 1.

Table I. *Total yellow colour of the butter from groups of Shorthorn cows on a normal winter ration, and on rations containing varying amounts of artificially dried grass.*

Stated as Lovibond units.

Week ending	D1 Control	E1 (10 % dried grass)	E2 (25 % dried grass)	E3 (50 % dried grass)
17. xi. 32*	6·8	8·8	8·0	7·2
15. xii. 32	7·2	8·4	9·2	12·8
29. xii. 32	6·8	8·4	8·4	12·0
23. i. 33	6·0	7·6	7·6	10·0
13. ii. 33	4·4	6·0	6·0	9·2
2. iii. 33	4·8	6·4	5·2	8·4
27. iii. 33	5·2	6·0	5·2	9·6
Averages	5·7	7·1	6·9	10·3

* All four groups on control ration.

The total yellow colour of the butter has not been markedly affected by the inclusion of 10 and 25 % of artificially dried grass in the concentrate ration, but the group receiving 50 % of its concentrates in the form of this material shows a much greater improvement in this respect.

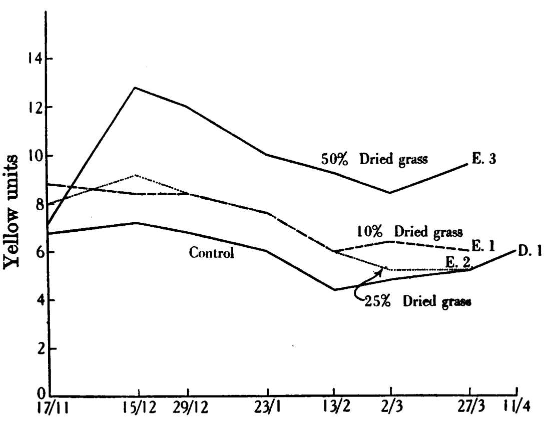

Fig. 1.

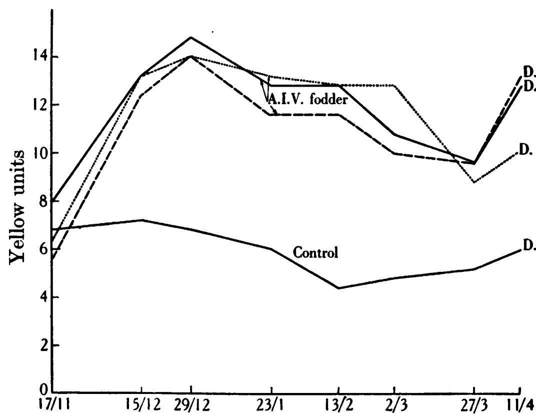

Fig. 2.

In the next experiment four pairs of cows were selected from the Shorthorn herd. One of these (group D1 *supra*) received an ordinary winter ration of hay, roots and concentrates, group D2 had a part of these replaced by 40 lb. of A.I.V. fodder, D3 by 70 lb. of A.I.V. fodder, whilst D4 received no purchased foodstuffs but only A.I.V. fodder, hay and artificially dried grass, all four rations being of equivalent nutritive value (starch equivalent and protein equivalent).

A.I.V. fodder is produced by a modification of the ensilage process in which a forage crop—grass in this case—is treated with a dilute solution of mineral acids in such amounts that an acidity of p_H 3–4 is developed in the mass, which is stored in a suitable container to which the free access of air is prevented [Virtanen, 1933]. The temperature of the mass does not rise appreciably, and the carotene content of the green crop is retained in a large measure, whereas in the case of the warm fermentation process of ensilage, in which the temperature rises to the neighbourhood of 50°, most of the pigment is destroyed.

The test continued throughout the winter and the results are given in Table II in terms of the total yellow colours of the butters made from the milk.

Table II. *Total yellow colour of the butter from groups of Shorthorn cows on a normal winter ration, and on rations containing varying amounts of A.I.V. fodder.*

Stated as Lovibond units.

Week ending	D 1 Control	D 2 (40 lb. A.I.V.)	D 3 (70 lb. A.I.V.)	D 4 (70 lb. A.I.V. + dried grass)
17. xi. 32*	6·8	8·0	5·6	6·4
15. xii. 32	7·2	13·2	12·4	13·2
29. xii. 32	6·8	14·8	14·0	14·0
23. i. 33	6·0	12·8	11·6	13·2
13. ii. 33	4·4	12·8	11·6	12·8
2. iii. 33	4·8	10·8	10·0	12·8
27. iii. 33	5·2	9·6	9·6	8·8
11. iv. 33	6·0	12·8	13·2	10·0
Averages	5·8	12·4	11·8	12·1

* All four groups on control ration.

These results are also shown graphically in Fig. 2. The control group D1 produced butters which were pale in colour until the cows were turned out to pasture in April, when the colour value began to rise. The feeding of 40 lb. of A.I.V. fodder greatly increased the total yellow colour of the butter, whilst the 70 lb. of A.I.V. fodder were of equal value in this regard, but no better than 40 lb. Group D4 (A.I.V. fodder, artificially dried grass and hay only) showed no further advantage over the other two experimental groups. The rise in colour in April coincided with the advent of fresh grass.

Table III. *Carotene contents of A.I.V. fodder and artificially dried grass.*

Stated as mg./100 g. of the dry weight.

Date	A.I.V. fodder	Date	Artificially dried grass
23. xi. 32	52·2	6. xii. 32	47·9
13. xii. 32	33·9	20. xii. 32	38·0
11. i. 33	44·1	28. xii. 32	35·9
24. i. 33	38·1	11. i. 33	35·2
28. ii. 33	47·2	26. i. 33	40·1
21. iii. 33	40·8	6. ii. 33	34·4
—	—	10. ii. 33	46·9
—	—	16. ii. 33	36·0
—	—	24. ii. 33	26·6
—	—	3. iii. 33	26·7
—	—	10. iii. 33	28·4
—	—	16. iii. 33	34·5
Average	42·7	Average	35·9

The comparative effects of the rations of dried grass and of A.I.V. fodder in these experiments are shown in Fig. 3, where the group receiving 50 % of its concentrate ration as dried grass (E3) is compared with that receiving 40 lb. of A.I.V. fodder (D2) and with the control group (D1) in all of which the total nutrient values (S.E. and P.E.) of the rations were equal. The A.I.V. fodder has been more effective, but throughout the trial the cows on this diet were receiving a greater amount of dry matter and of carotene daily in the form of A.I.V. fodder than was fed in the form of artificially dried grass. The A.I.V. fodder supplied on the average 10 lb. of dry matter per head daily, whereas the dried grass supplied about 6 lb.

The carotene contents of the two foodstuffs were determined at intervals and the results are given in Table III.

In another experiment four pairs of Ayrshire cows were used (F1 to F4). The control group F4 received a normal winter ration similar to that fed to group D1 of the Shorthorns. To groups F1 and F3 were fed 40 lb. per head daily of A.I.V. fodder made with different acid mixtures, whilst group F2 received 40 lb. per head daily of a fodder made by the addition of a weaker acid mixture, sufficient to raise the acidity of the mass only to p_H 4·5, together with some molasses to stimulate a lactic acid fermentation.

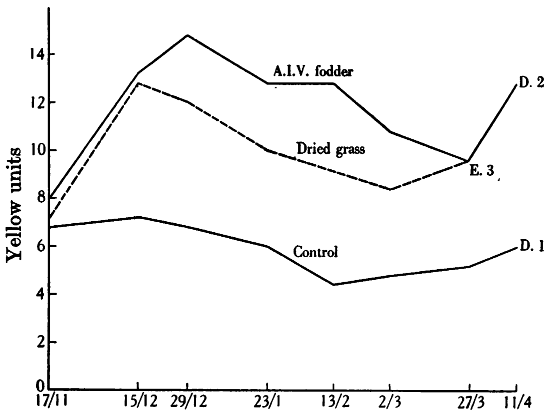

Fig. 3.

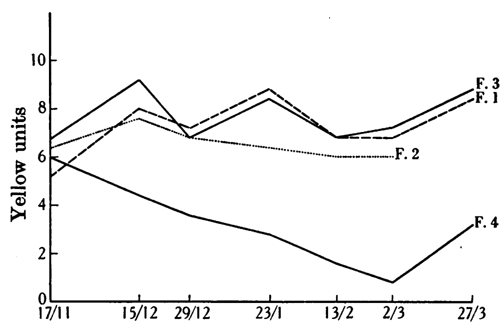

Fig. 4.

The results of this experiment are given in Table IV in terms of the total yellow colour of the butters.

Table IV. *Total yellow colour of the butter from groups of Ayrshire cows on a normal winter ration and on rations containing different types of acid-treated green fodder.*

Week ending	F1 (A.I.V. fodder No. 1)	F2 (Acid + molasses fodder)	F3 (A.I.V. fodder No. 2)	F4 Control
17. xi. 32*	5·2	6·4	6·8	6·0
15. xii. 32	8·0	7·6	9·2	4·4
29. xii. 32	7·2	6·8	6·8	3·6
23. i. 33	8·8	6·4	8·4	2·8
13. ii. 33	6·8	6·0	6·8	1·6
2. iii. 33	6·8	6·0	7·2	0·8
27. iii. 33	8·4	—	8·8	3·2
Averages	7·7	6·6	7·9	2·7

* All four groups on control ration.

The values are shown graphically in Fig. 4. The effect of the inclusion of a foodstuff rich in carotene is again apparent in the total yellow colour of the butter. The two A.I.V. fodders show some advantage in this respect over the fodder treated with a weaker acid mixture (fed to group F2) but in this case the fodder was made from grass of poorer quality than the other two types.

The carotene contents of the three fodders used are given in Table V; they account for the somewhat smaller effect obtained with the fodder made with a lower addition of acid. The supply of this latter fodder was exhausted in early March, and this group was then discontinued.

Table V. *Carotene contents of fodders fed to the groups of Ayrshire cows.*

(Stated as mg./100 g. of the dry matter.)

Date	F 1	F 2	F 3
13. xii. 32	56·7	33·3	64·6
11. i. 33	53·9	29·5	67·2
24. i. 33	50·4	25·2	56·1
9. ii. 33	38·5	47·4	42·8
21. ii. 33	28·8	35·6	59·5
21. iii. 33	41·4	—	58·2
Averages	44·9	34·2	58·1

An interesting point is the difference in level of the maximum yellow colour value of the butter in the Ayrshire groups as compared with the Shorthorns. With the former breed it scarcely exceeded 9 Lovibond units, whereas with the Shorthorns it rose to almost 15.

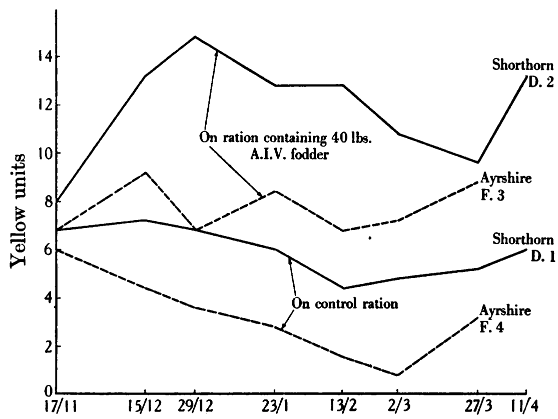

Fig. 5.

This difference with breed is shown clearly in Fig. 5, where the values for the two control groups, Shorthorn and Ayrshire, are plotted together with the values obtained when 40 lb. of A.I.V. fodder were fed (groups D2 and F3). It is noticeable that the Ayrshire butter lost its colour more rapidly and more completely than the Shorthorn on the same normal winter ration.

When a foodstuff rich in carotene was included in the ration the yellow colour of the butter was increased, but to a far greater extent with the Shorthorn cows. It seems that there is, for each breed, a "ceiling" value above which the depth of colour of the butter fat does not rise even when an excess of fodder rich in carotene is fed.

This ceiling value for the Ayrshire cows seems to be markedly lower than it is for the Shorthorns. Values obtained on the farm herds in June 1933, when the pasture was at its best, gave values of 15·2 Lovibond units for Shorthorn butter and 9·2 for Ayrshire butter, which agree well with the maximum values obtained on the A.I.V. fodder rations during the winter (14·8 and 9·2 respectively).

The difference in intensity of colour with breed has been shown by many workers, and it is generally known that the Channel Island breeds in Jersey and Guernsey produce a milk fat of higher colour than all the others, and that the Ayrshire falls below the other breeds in that regard. Wilbur *et al.* [1933] have shown that despite the difference in yellow colour, the vitamin A potency of the butter made from the milk of Jersey cows is no greater than that of Ayrshires. There are indications also from the values obtained from the butter of pure-bred Ayrshires that the vitamin A potency is similar to that of Shorthorn butter.

It would appear that the total yellow colour of butter from any one breed of cow is correlated with its carotene content and its vitamin A content, but that the depth of colour is no indication of the total vitamin A potency of the butter unless the breed from which the milk was obtained is known. A comprehensive experiment, including the major dairy breeds, is being conducted at present to obtain full data on this point.

Although the experiments were not designed to measure the effect of the various rations on the yield of milk, all yields were normal and the performance of all the cows was satisfactory.

Two trials of a different type were carried out at outside centres, the first at Dartington Hall in Devonshire[1], the other in Derbyshire. Thirteen South Devon cows were used at the former centre and eight Shorthorn cows in Derbyshire. All the cows at each centre were fed on a normal winter ration of concentrates for 5 weeks. They were all then changed over to an experimental ration containing 40 lb. of A.I.V. fodder per head daily, the nutritive value of the diet being the same as in the first period. After 5 weeks on this diet they were again changed back to the control ration, which was identical with that fed in the first period. The final period also lasted 5 weeks. The milk was sampled in the fifth week of each period, and butter samples were made and their total yellow colour was measured (Table VI).

Table VI. *Total yellow colour of the butter produced by two groups of cows on a normal winter ration and on one containing 40 lb. of A.I.V. fodder.*

Stated in Lovibond units.

	Period 1 Control	Period 2 A.I.V. fodder	Period 3 Control
South Devon cows	4·0	8·4	2·8
Shorthorn cows	2·0	8·4	2·4

The effect of the A.I.V. fodder on the total yellow colour of the butter is striking. The colour value is not so high as that obtained on 40 lb. of the A.I.V. fodder at Jealotts Hill, but it must be remembered that the experimental ration was fed for only 5 weeks and that the cows had been on a winter diet for 5 weeks, which had reduced the colour to a low level. It is a point of great practical interest that it is possible to raise the yellow colour of the milk fat so rapidly from a low level. The return of the colour to its previous low level

[1] A full account of this trial is being published elsewhere.

after reverting to the control ration for 5 weeks is indicative of the necessity for including a foodstuff rich in carotene in the diet of the dairy cow throughout the whole winter period if the colour of the milk and its quality are to be held at the high level which is so desirable from a nutritional standpoint.

II. THE CAROTENOID AND VITAMIN A CONTENTS OF THE BUTTER.

The preceding section of this paper describes the results obtained using as criterion the colour of the butter produced by the various experimental groups. The present section, on the other hand, gives the results of the spectroscopic examination of certain of the butters, with particular reference to the carotene, xanthophyll and vitamin A contents, determined by the method developed earlier [Gillam *et al.*, 1933]. The numerical results seen in Table VII correspond to the experiment described on p. 1077 (*cf.* also Table II).

Table VII.

				Constituents as mg./100 g. of dry butter			
Group	Ration	Date of milk sample	H_2O in butter	Carotene (1900 basis)	Xanthophyll (1700 basis)	Vit. A by gross absorption 328 $m\mu$ (1600 basis)	Vit. A by absorption at 585 $m\mu$ in $SbCl_3$ colour test (2600 basis)
D 1	Control	16. i. 33	18·5	0·040	0·0186	0·292	0·222
		6. ii. 33	13·9	0·146	0·0144	0·298	0·157
		27. ii. 33	12·6	0·164	0·009	0·286	0·122
		20. iii. 33	17·9	0·025	0·0036	0·268	0·118
D 2	40 lb. A.I.V. fodder per cow per day	17. i. 33	16·0	0·607	0·065	0·63	0·480
		7. ii. 33	9·5	0·188	0·023	0·519	0·259
		28. ii. 33	12·7	0·347	0·039	0·494	0·196
		21. iii. 33	17·8	0·164	0·0134	0·407	0·281
		11. iv. 33	16·0	0·170	0·014	0·715	0·293
D 3	70 lb. A.I.V. fodder per cow per day	18. i. 33	12·8	0·543	0·063	0·372	0·294
		8. ii. 33	13·9	0·448	0·022	0·428	0·194
		1. iii. 33	17·0	0·325	0·0114	0·482	0·270
		22. iii. 33	19·1	—	0·026	0·430	0·169
		12. iv. 33	17·2	0·152	0·012	0·693	0·333
D 4	70 lb. A.I.V. fodder + 4 lb. dried grass per cow per day	18. i. 33	14·3	0·638	0·029	0·57	0·382
		9. ii. 33	12·7	0·362	0·043	0·575	0·341
		2. iii. 33	11·4	0·386	—	0·442	0·207
		22. iii. 33	12·4	0·388	0·0314	0·592	0·385
		13. iv. 33	15·6	—	—	0·578	0·241

The figures stated as the basis of calculation indicate the value of $E^{1\%}_{1\,\text{cm.}}$ taken as that of each of the pure substances. ($E = \log I_0/I$ for a 1 cm. layer of a 1 % solution, where I_0 = the intensity of the incident light and I = the intensity of the transmitted light at the wave-length of maximum absorption characteristic of each substance.)

The mean values for each of the groups over the whole period of the experiment are shown in Table VIII and clearly demonstrate the effect of the diet rich in carotene on the vitamin A and carotene contents of the butters.

A further experiment to test the value of A.I.V. fodder was carried out at Dartington Hall (Devonshire) using a single group of 13 pure-bred South Devon

Table VIII.

	Carotene mg./100 g. dry weight	Xanthophyll mg./100 g. dry weight	Vitamin A mg./100 g. dry weight U.V. values
D 1. Control	0·094	0·0114	0·286
D 2. 40 lb. A.I.V.	0·295	0·0308	0·553
D 3. 70 lb. A.I.V.	0·367	0·027	0·481
D 4. 70 lb. A.I.V. + 4 lb. dried grass	0·428	0·034	0·551

cows (see p. 1081). The results of the spectroscopic determinations are given in Table IX and, together with the evidence of the total yellow colour values given earlier, leave no doubt as to the value of A.I.V. fodder in the diet of the cow as a means of increasing the carotenoid and vitamin A contents of winter butter.

Table IX.

	First period	Second period	Third period
Ration	Control (5 weeks)	A.I.V. fodder (5 weeks)	Control (5 weeks)
Date of sample	25. i. 33	1. iii. 33	7. iv. 33
H_2O, %	13·7	12·1	12·6
Carotene, mg./100 g. of dry wt. (1900 basis)	0·122	0·311	0·129
Xanthophyll, mg./100 g. of dry wt. (1700 basis)	0·0081	0·0366	0·0148
Vitamin A, mg./100 g. of dry wt. By 328 $m\mu$ band (1600 basis)	0·183	0·405	0·240

The colour of cow's butter is known to vary with different breeds, but as the absence of yellow colour (or carotene) from butter does not necessarily imply the absence of the true vitamin A also, it was decided to examine a series of goat butters—which are noted for their chalky-white colour and low carotenoid content. Monthly samples were therefore obtained from one particular herd over the period June-December inclusive. The unsaponifiable matter was a much paler yellow than that of cow butter, and in chloroform solution it exhibited ill-defined maxima near 480 and 455 $m\mu$. As only a small fraction of the already weak yellow colour was extractable from light petroleum solution by methyl alcohol the amount of xanthophyll present must have been very small [Willstätter and Stoll, 1913]. The yellow colour remaining in the petroleum showed ill-defined absorption bands identical with those characteristic of the carotene fraction of cow's butter.

With antimony trichloride in chloroform the unsaponifiable matter gave a deep blue colour, much stronger than that to be expected from the small content of carotenoids. The blue solution exhibited two maxima at 612–618 $m\mu$ and 580–586 $m\mu$ characteristic of vitamin A, the presence of which was confirmed by the occurrence of an inflexion in the absorption curve of the chloroform solution near 328 $m\mu$.

Monthly samples (250 g.) were examined by the method applied to the cow's butter and the numerical results are given in Table X.

Vitamin A with antimony trichloride gives a blue colour exhibiting a strong absorption band at 617 $m\mu$ and a masked maximum close to 583 $m\mu$. The relative intensities of these bands are approximately 2 : 1 [Heilbron *et al.*, 1932; Carr and Jewell, 1933]. In cod-liver oil, however, the relative intensity of the 617 $m\mu$ band is considerably reduced presumably owing to the presence of certain inhibiting substances [Norris and Church, 1930]. Artificial inhibitors of this type, mostly decomposition products of proteins, have been described by

Table X. *Goat's butter.*

Date	H_2O %	Constituents expressed as mg./100 g. dry butter: Carotene* by light absorption at 460 $m\mu$	Vitamin A by 328 $m\mu$ band (1600 basis)	Vitamin A by 585 $m\mu$ band (in $SbCl_3$ blue) (2600 basis)
June	14·5	—	0·239	0·22
July	14·9	0·017	0·564	0·43
August	12·0	0·026	0·454	0·27
September	12·9	0·014	0·393	0·30
October	13·9	0·012	0·341	0·33
November	11·6	0·023	0·420	0·34
December	13·1	0·036	0·450	0·30

* Carotene values uncorrected for xanthophyll.

Table XI. *Comparison of goat's and cow's (pure-bred Shorthorn) butter.*

	Constituents expressed as mg./100 g. dry butter: Carotene	Vitamin A
Goat's butter. Mean values (June-December)	0·021	0·41
Cow's butter. Cows on ordinary winter diet (typical values)	0·15	0·28
Cow's butter. Cows on rich winter diet (typical values)	0·35	0·55
Cow's butter. Cows at grass (moderate summer values)	0·60	0·70

Emmerie *et al.* [1931], and the action of one of them—7-methylindole—on the vitamin A-antimony chloride blue colour has been examined fully [Morton, 1932]. The unsaponifiable matter of cow's butter must also contain these or similar inhibiting substances for with antimony trichloride in chloroform the intensity of the 617 $m\mu$ band is only the same or slightly more than that of the 583 $m\mu$ band and even the intensity of the latter band is reduced. This is also shown by the fact that the vitamin A values calculated from the intensity of this band are much lower than those obtained by the ultra-violet absorption at 328 $m\mu$. The distinct nature of this inhibition will be seen by a comparison of the vitamin A values measured by the two methods (Table VII). The inhibition of these absorption bands in the blue antimony trichloride solution of the unsaponifiable matter of butter is probably due to the same cause as that which gives the low blue colour values (Lovibond) on butter fat reported by Booth *et al.* [1933], who find the amount of inhibition to vary seasonally. In the samples of the unsaponifiable matter of goat's butter we find that although both absorption bands are reduced in intensity the inhibition is not so marked as in the case of cow's butter. This is shown by the fact that the vitamin A values of goat's butter, calculated from the intensity of the 585 $m\mu$ absorption band, do not differ quite so much as in the case of cow's butter from those obtained by the ultra-violet absorption method (Table X).

Summary.

The effect of a ration containing artificially dried grass on the quality of the milk of Shorthorn cows was tested. When 10 or 25 % of the concentrated foodstuffs fed in the production ration were replaced by artificially dried grass no appreciable advantage in depth of yellow colour of the butter-fat was noted, as compared with a control group on a normal winter ration. The inclusion of

50 % of artificially dried grass in the concentrated ration, however, caused a distinct improvement in the yellow colour of the butter fat.

The inclusion of A.I.V. fodder in the winter ration of Shorthorn cows raised the yellow colour of the butter-fat to a high level, comparable with that obtained on best pasture. The A.I.V. fodder was fed at two levels, 40 lb. and 70 lb. per head daily. The larger amount showed no advantage, either in colour or vitamin A content of butter fat, over an allowance of 40 lb. per head daily.

The feeding of A.I.V. fodder increased the carotene and xanthophyll contents of the resulting butter to about three times those of a similar control group on a normal winter ration. The content of the true vitamin A was approximately doubled at the same time.

The feeding of A.I.V. fodder to Ayrshire cows raised the yellow colour of the butter-fat to a level well above that from milk produced by a group on a normal winter ration.

The difference in the depth of yellow colour of butter fat as between breeds, is well marked in the case of Shorthorn and Ayrshire cows. On a normal winter ration deficient in carotene, the yellow colour of the butter fat of Ayrshire cows fell more rapidly and to a decidedly lower level than was the case with Shorthorn cows. There seems to be, for each of these two breeds, a "ceiling" value above which the colour intensity and vitamin A content of the butter fat do not rise, even when an excess of carotene-rich fodder is fed.

The feeding of 40 lb. of A.I.V. fodder in the ration raised the yellow colour of the butter fat of Shorthorn cows to a high "ceiling" level, whereas with the Ayrshire the "ceiling" value was much lower, though the amount of carotene supplied in the ration was similar for both breeds. In spite of this difference in depth of colour, it appears that there may be little difference in the content of true vitamin A in the butters from the two breeds.

Samples of goat's butter were found to be very similar to cow's butter in their content of the true vitamin A, although they were almost devoid of colour; the carotene content was less than 5 % of that of the butter from cows at grass. Thus the colour of goat's butter cannot be used as an indication of its growth-promoting activity in comparison with cow's butter, since in goat's butter most of this activity is due to the presence of colourless preformed vitamin A and not to carotene.

The thanks of the authors are due to the Research Council of Messrs Imperial Chemical Industries, Ltd. for a grant which has defrayed part of the expenses of this work, to Mr W. S. Ferguson and other members of the staff there for their assistance in the work carried out at Jealotts Hill, and to Mr H. J. Page, under whose direction that work was carried out.

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